Remarks

These amendments simply correct typographical errors and add no new matter to the specification.

The amendments made on page 1, line 30; and page 7, line 12; and page 8, line 5 were made to correct the page number of the document cited. The authors, journal titles, volume numbers, and years of publication were all cited correctly, and from this information the correct page numbers may be easily found.

The amendments made on page 2, line 6; page 8, line 2; page 35, line 24; and page 35, line 27 were made to correct the spelling of the first-named authors of the cited documents. The journal titles, volume numbers, page numbers and years of publication were all cited correctly, and from this information the correct spelling of the first-named authors could easily be found.

The amendments made on page 7, line 21 were made to correct the journal title (removing the unnecessary words) and to corect the page numbers. The authors, part of the journal title, volume number, and year of publication were all cited correctly, and from this information the correct journal title and page numbers may be easily found.

The amendment on page 7, line 32 was made to correct the volume number, and page number of the cited document. The authors, journal title and year were correctly cited, and a search of the literature by author and journal title and year, taken in the context of the passage in the specification where the citation occurs, would enable one skilled in the art to determine the correct citation of the document.

The amendments made on page 8, line 7, were made to correct the journal title and page numbers of the document cited. The author, volume number, and year of publication were cited correctly. A search of the literature, such as a search of the PubMed database on the National Institutes of Health website, by author, volume number, and year yields the correct citation without difficulty.

The amendment made on page 8, line 24, corrects the citation for the catfish I sequence to read Minth et al. The Minth et al. document was cited for the catfish I sequence in the originally filed application at page 19, line 28.

The amendments made on page 12, line 29 and page 13, line 4 were made to correct the name of the author of the same citation. The journal title, volume number, page numbers, and

year of publication are all correct. Further, the name mistakenly cited was the first name of the author of the document cited.

The amendments made on page 12, line 31 and page 13, line 5 were made to correct the Internet website address for the document cited. One skilled in the art would be familiar with this website and easily determine the correct website.

The amendment made to page 26, line 21 merely completes the citation by adding the author's name. A search of the literature by book title yields the correct citation without difficulty.

The amendment to page 30, line 12 adds the year of publication. The authors, journal title, volume number, and page numbers were all cited correctly, and from this information the correct year of publication may be easily found. In addition, this reference is correctly identified at page 8, line 26 of the specification.

The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

CERTIFICATE UNDER 37 C.F.R. 1.8:

The undersigned hereby certifies that this paper is being deposited in the United States Postal Service, as first class mail, in an envelope addressed to: Assistant Commissioner for Patents, Washington, D.C. 20231, on this 9th day of July, 2001.

Victoria A. Sandberg

July 9, 2001
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Appendix A to Second Preliminary Amendment

Specification Amendments with Notations to Indicate Changes Made
Applicants: Mark A. Sheridan et al.
Serial No.: 09/727,739
Filed: December 1, 2000

For: SOMATOSTATINS AND METHODS

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted. Additionally, all amendments have been shaded.

Page 1, line 16 to page 2, line 9

Somatostatins are ubiquitous polypeptides known to affect basic biological processes such as growth, development, metabolism, and cell differentiation in vertebrates. Somatostatin was first isolated as a 14-amino acid peptide from ovine hypothalamus and found to inhibit the release of growth hormone from the pituitary gland (Brazeau et al., Science, 179, 77-79 (1973)). Since then somatostatins have been isolated from representatives of nearly every major group of vertebrates examined to date, from jawless fish to mammals (Conlon et al., Regul. Peptides, 69, 95-103 (1997)). Somatostatins have been found broadly in the central (*e.g.*, cerebral cortex, cerebellum, pineal, olfactory lobe, hypothalamus, spinal cord) and peripheral nervous systems, gastrointestinal tract (*e.g.*, salivary glands, stomach, intestine), urogenital tract (*e.g.*, bladder, prostate, collecting ducts of the kidney), pancreatic islets, adrenal glands, thyroid tissue, and placenta as well as in cerebral spinal fluid, blood, and saliva (Reichlin, "Somatostatin," Brain peptide; Krieger et al., eds., John Wiley and Sons, New York, pp. [712] 711-752 (1982); Gerich, "Somatostatin and analogues," Diabetes mellitus: Theory and practice, Ellenberg et al., eds., Medical Examinations, New York (1983); Wass, "Somatostatin," Endocrinology, DeGroot, ed., WB Saunders, Philadelphia, PA (1989); Patel, "General aspects of the biology and function of somatostatin," Basic and clinical aspects of neuroscience, Weil et al., eds., Springer-Verlag, Berlin (1992)). In neurons and cells, somatostatins are often co-localized with other factors (*e.g.*, norepinephrine, CCK, neuropeptide-Y, CGRP, GABA, VIP, substance P) ([Gibbons] Gibbins, "Co-existence and co-function," The comparative physiology of regulatory peptides, Holmgren, ed., Chapman and Hall, London/New York (1989)).

Page 7, line 5 to line 16

Figure 5 compares the amino acid (lower left) and cDNA nucleotide (upper right) sequence identities vertebrate somatostatins. AF I denotes anglerfish (Hobart et al., Nature, 288, 137-141 (1980)), CF I denotes catfish I (Minth et al., J. Biol. Chem., 257, 10372-10377 (1982)), H denotes human (Shen et al., Proc. Natl. Acad. Sci. USA, 79, 4575-4579 (1982)), B denotes bovine (Su et al., Mol. Endocrinol., 2, 209-216 (1988)), M denotes monkey (Travis and Sutcliffe, Proc. Natl. Acad. Sci. USA, 85, 1696-1700(1988)), R denotes rat (Goodman et al., J. Biol. Chem., 258, [570-573] 5570-5573 (1983)), C denotes chicken (Nata, GenBank direct submission, Accession No. X60191 (1991)), FR I denotes frog (Tostivint et al., Proc. Natl. Acad. Sci. USA, 93, 12605-12610 (1996)), TR II' denotes rainbow trout-II' (Moore et al., Gen. Comp. Endocrinol., 98, 253-261 (1995)), TR II" denotes rainbow trout-II", and TR I denotes rainbow trout-I.

Page 7, line 17 to page 8, line 10

Figure 6 aligns the deduced rainbow trout PPSS-I C-terminal region amino acid sequence to other PPSS-I C-terminal region amino acid sequences from other vertebrates. [a]Sequences arranged for maximum alignment; identity is greatest if it is assumed there has been a 2-amino acid deletion (designated by asterisks) from rainbow trout and bowfin (Wang et al., [Amia calva.] Regul. Peptides, 47, 33-[40] 39 (1993). [b]Putative peptide deduced from cDNA. [c]Peptide sequence deduced from cDNA and confirmed by processing analysis for anglerfish I (Hobart et al, Nature, 288, 137-141 (1980); Goodman et al., Proc. Natl. Acad. Sci. USA, 77, 5869-5873 (1980); Andrews and Dixon, Biochemistry, 26, 4853-4861 (1987)), catfish I (Andrews and Dixon, J. Biol. Chem., 256, 8267-8270 (1981); Minth et al., J. Biol. Chem., 257, 10372-10377 (1982)), and frog (Vaudry et al., Biochem. Biophys. Res. Commun., 188, 477-482 (1992); Tostivint et al., Proc. Natl. Acad. Sci. USA, 93, 12605-12610 (1996)). [d]Peptide sequence derived directly from analysis of isolates of islet extracts obtained from hagfish (Conlon et al., Endocrinology, 122, 1855-1859 (1988)), lamprey (Andrews et al., J. Biol. Chem.,[258, 5570-5573] 263, 15809-15814 (1988)), torpedo (Conlon et al., Gen. Comp. Endocrinol., 60, 406-413 (1985)), ratfish (Conlon et al., Gen. Comp. Endocrinol., 80, 314-320 (1990)), sturgeon ([Nishi] Nishii et al., Gen. Comp. Endocrinol., 99, 6-12 (1995)), eel (Conlon et al., Endocrinology, 122, 1855-1859 (1988)), flounder and sculpin (Conlon et al., Eur. J. Biochem., 168, 647-652 (1987a)), salmon (Plisetskaya et al., Gen. Comp. Endocrinol., 63, [242] 252-263 (1986)), salamander (Cavanaugh et al., Gen. Comp. Endocrinol., 101, 12-20 (1996)), pigeon (Spiess et al., [Endocrinology, 76, 33-40] Proc. Natl. Acad. Sci. USA, 76, 2974-2978 (1979)), alligator (Wang and Conlon, Peptides, 14, 573-579 (1993)), and ovine (28-amino acid form shown for purposes of comparison; Pradayrol et al., FEBS Lett., 109, 55-58 (1980)).

Page 8, line 11 to line 32

Figure 7 aligns the deduced rainbow trout PPSS-I, PPSS-II' and PPSS-II" amino acid sequences with PPSSs of other vertebrates; sequence identity was maximized by inserting gaps (denoted by dashed lines); conserved amino acids are shaded. H denotes human (Shen et al., Proc. Natl. Acad. Sci. USA, 79, 4575-4579 (1982)); M denotes monkey (Travis and Sutcliffe, Proc. Natl. Acad. Sci. USA, 85, 1696-1700 (1988)); B denotes bovine (Su et al., Mol. Endocrinol., 2, 209-216 (1988)); R denotes rat (Goodman et al., J. Biol. Chem., 258, 570-573 (1983)); C denotes chicken (Nata, GenBank direct submission, Accession No. X60191 (1991)); FR I and FR II denote frog I and frog II (Tostivint et al., Proc. Natl. Acad. Sci. USA, 93, 12605-12610 (1996)); AF I denotes anglerfish I (Hobart et al., Nature, 288, 137-141 (1980)); AF II denotes anglerfish II (Goodman et al., Proc. Natl. Acad. Sci. USA, 77, 5869-5873 (1980); Goodman et al., Proc. Natl. Acad. Sci. USA, 79, 1682 (1982); Hobart et al., Nature, 288, 137-141 (1980)); CF I denotes catfish I ([Eilertson and Sheridan, Gen. Comp. Endocrinol., 92, 62-70 (1993)] Minth et al., J. Biol. Chem., 257, 10372-10377 (1982)); CF II denotes catfish II (Fujita et al., Peptides, 2, 123-131 (1981)); GF I-III denotes goldfish I-III (Lin et al., Endocrinology, 140, 2089-2099 (1999)); TRI denotes trout I; TRII' denotes trout II' (Moore et al., Gen. Comp. Endocrinol., 98, 253-261 (1995)); and TRII" denotes trout II".

Page 12, line 22 to page 13, line 8

Percent identity is determined by aligning the residues of the two amino acid or nucleotide sequences to optimize the number of identical amino acids or nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical amino acids or nucleotides, although the amino acids or nucleotides in each sequence must nonetheless remain in their proper order. Preferably, two amino acid sequences are compared using the Blastp program, version 2.0.9, of the BLAST 2 search algorithm, as described by [Tatiana] Tatusova et al. (*FEMS Microbiol. Lett.*, 174, 247-250 (1999)), and available at [http://www.ncbi.nlm.nih.gov/blast.html] <http://www.ncbi.nlm.nih.gov/blast/>. Preferably, the default values for all BLAST 2 search parameters are used, including matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x_dropoff = 50, expect = 10, wordsize = 3, and filter on. Likewise, two nucleotide sequences are compared using the Blastn program, version 2.0.11, of the BLAST 2 search algorithm, also as described by [Tatiana] Tatusova et al. (*FEMS Microbiol Lett*, 174, 247-250 (1999)), and available at [http://www.ncbi.nlm.nih.gov/blast.html] <http://www.ncbi.nlm.nih.gov/blast/>. Preferably, the default values for all BLAST 2 search parameters are used, including reward for match = 1, penalty for mismatch = -2, open gap penalty = 5, extension gap penalty = 2, gap x_dropoff = 50, expect = 10, wordsize = 11, and filter on.

Page 26, line 17 to line 26

Oligonucleotides were either custom synthesized by National Biosciences (Plymouth, MN) or supplied with Gibco/BRL 3'- and 5'-RACE kits. Oligonucleotides used as probes were 5'-end labeled with [γ^{32}P]-ATP (Amersham) using T4-polynucleotide kinase (Promega) as previously described in Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edition, Plainview, New York, Cold Spring Harbor Laboratory Press (1989). The full-length SS-II cDNA probe was radiolabeled with [α^{32}P]-CTP by random priming (Prime-a-Gene; Promega) according to the manufacturer's protocol. All radiolabeled probes were purified over Elutip-D columns (Schleicher and Schuell) according to the manufacturer's protocol.

Page 30, line 7 to line 22

The deduced PPSS-II' (SEQ ID NO:9) and PPSS-II" (SEQ ID NO:15) proteins in rainbow trout Brockmann bodies contain 115 and 111 amino acids, respectively, both slightly shorter than the precursors of anglerfish (Goodman et al., J. Biol. Chem., 258, 570-573 (1983); Goodman et al., Proc. Natl. Acad. Sci. USA, 77, 5869-5873 (1980); and Hobart et al., Nature, 288, 137-141 (1980)), and goldfish (Lin et al., Endocrinology, 140, 2089-2099 (1999)), the only other known PPSS-IIs containing [Tyr^7, Gly^{10}]-SS-14. Rainbow trout PPSS-II' shared 43.5 % amino acid identity with anglerfish PPSS-II and 51.3 % amino acid identity with goldfish PPSS-II. The amino acid identity between rainbow trout PPSS-II" and anglerfish PPSS-II was 38.7 % while the identity between trout PPSS-II" and goldfish PPSS-II was 41.4%. Amino acid identities between rainbow trout PPSS-IIs and precursors derived from gene 1 were lower, between 37.9 % and 22.5 %. Rainbow trout PPSS-IIs were least similar to the preprosomatostatin giving rise to catfish SS-22. Although the evidence is limited, it appears that evolutionary selection has acted to conserve the biologically active C-terminal domain of PPSSs (see Fig. 7).

Page 35, line 17 to page 36, line 4

The present study revealed that two PPSS-II mRNAs of rainbow trout are differentially expressed. This conclusion is based on several observations. First, the pattern of PPSS-II' mRNA and PPSS-II" mRNA is tissue-specific. For example, only PPSS-II" mRNA was detected in the brain of rainbow trout, whereas both PPSS-II' and PPSS-II" mRNA were detected in pancreas and various regions of the gut. Brain-specific expression of the mRNA encoding the alternate form of SS in frogs (denoted PSS2) (Tostivint et al., Proc. Natl. Acad. Sci. USA, 93, 12605-12610 (1996)) and cortistatin ([DeLecea] de Lecea et al., Nature, 381, 242-245 (1996)) also has been reported. Previous immunocytochemical studies support a similar distribution of [Tyr^{7},Gly^{10}]-somatostatin-14-containing peptides in the intestine ([Beorlegegui] Beorlegui et al., Gen. Comp. Endocrinol., 86, 483-495 (1992)) and stomach (Barrenechea et al., Tissue Cell, 26, 309-321 (1994)) of rainbow trout. Second, the abundance of PPSS-II mRNAs was different with specific tissues. Within the Brockmann body of rainbow trout, the predominant message form was that encoding for PPSS-II", whereas in the stomach the predominant form was that encoding PPSS-II'. Lastly, the pattern of PPSS-II expression within the endocrine pancreas of rainbow trout was modulated by nutritional state. Together, these results suggest that rainbow trout produce two forms of gene 2 SS peptides and that there exists mechanisms to independently regulate the expression of each.